Red Clover Seed Production: V. Root Health and Crop Productivity

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ABSTRACT

Red clover (*Trifolium pratense* L.) is an important forage legume that is primarily grown for seed in western Oregon, but the effects of root health on red clover seed production systems are not well defined. This study was conducted to determine the effects of root health on red clover seed production. Thirty-one seed fields were selected in spring 1992 and their cultivar identity, age of stand, and seed certification status (seed source groupings) determined by grower interviews and DNA analyses. Two herbage removal time treatments (early May and late June) were applied and the number of flowers and soil water content measured during the period of flowering and seed production. Root rot [*Fusarium solani* (Kuhn)] and root borer [*Hylastinus obscurus* (Marsham)] infestation were measured at early herbage removal time and seed harvest. The percentage of plants infested with root borers was the greatest root health determinate of seed yield, regardless of early or late herbage removal time. Seed yield was also correlated with the regrowth and flower production capacity of plants following herbage removal. For both herbage removal times, regrowth after removal was affected by the plant capacity to deplete soil water. Season-end root borer infestation and soil water depletion amount were inversely related, indicating root integrity affected water utilization. Second-year seed crops had greater disease and root borer damage than first-year crops. Late herbage removal time treatments reduced flower density, seed yield, and season-end phytomass compared with early removal. Genetic selection for improved root borer resistance may be a useful alternative selection strategy to root rot resistance for increasing red clover seed yields.

In the USA, Canada, and northern and eastern Europe, red clover is an important temperate forage legume crop that is used for hay, ensilage, and pasture. Successful red clover herbage and seed production systems depend on on the ability of plants to grow while exposed to various environmental stresses. Red clover is primarily grown as a specialty seed crop in the Willamette Valley of western Oregon, USA. This is in contrast to seed production in the midwestern USA where it is a source of revenue secondary to herbage production.

Oregon-adapted ecotypes are popular among seed growers because they generally have greater seed yields than improved cultivars. However, improved cultivars such as Marathon are desirable for herbage production because they have root rot resistance in forage production regions, produce more forage, and are more persistent (Steiner et al., 1997).

In northern U.S. states, root rot caused by *Fusarium oxysporum* Schlect. may severely injure red clover plants and seriously deplete or eliminate the stand after the first cutting (Taylor and Smith, 1995). Cultivars selected for resistance to *F. oxysporum* produced an average of 5.5 Mg ha^{-1} more herbage in 2 yr of production than three common ecotypes and 'Kenland' grown in Wisconsin (Steiner et al., 1997). However, root rot resistance and superior forage yield in midwestern U.S. cultivars does not necessarily result in greater seed yields in western Oregon seed production systems (Steiner et al., 1997). Also, the clover root borer can greatly reduce herbage yields (Pruess and Weaver, 1958; Jin et al., 1992) and intensify the severity of root rot (Leath and Byers, 1973; Leath et al., 1973).

The capacity of cultivars to flower after the time of herbage removal in spring is a greater determinate of final seed yield than root rot resistance (Steiner et al., 1997). Herbage removal time, cultivar genetic background, and the general productivity of a particular production site have been shown to influence red clover seed yields (Steiner et al., 1995). In the western Oregon red clover seed production region, root and crown rot diseases also cause reductions in seed yield, depending on the age of the stand and the amount of soil water that is available during periods of flowering and seed maturation (Oliva et al., 1994b). The effect of root disease infection and root borer infestation on seed yields have not been defined. This research identifies the primary causal agent of root disease and describes the effects of root rot disease infection and root borer infestation in relationship to time of herbage removal, age of stand, and genetic background on red clover seed production under the humid temperate marine climatic conditions found in western Oregon.

METHODS AND MATERIALS

Field Experiments

Without prior knowledge of soil type, age of stand, or cultivar, thirty-one commercial seed fields were selected throughout a seven-county area in the Willamette Valley region of western Oregon in the spring of 1992 (Table 1). The genetic sources of the plants grown at each site were determined by interviews with the seed growers. When named cultivars were reported, they were noted as being either certified or common seed classes. The location of each field was recorded with a global positioning system recorder.

Two herbage removal time treatments were applied at each location and were designated as early (7, 8, or 12 May) or late (22, 23, 24, or 26 June). Each plot was 4 by 4 m, the treatments were replicated three times, and were arranged in a randomized complete block design. A gasoline-powered mower was used to cut each plot to a ≈5-cm stubble height. The total amount of fresh-cut plant material from each plot was weighed and then 10 stems selected at random were weighed and put into plastic bags. The bags were transported to the laboratory where the 10-stem samples were transferred to paper bags and dried in a forced-air oven at 60°C for 24 h. The dried

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Abbreviations: loading values, factor analysis rotated loading values; PDA, potato dextrose agar; RAPD, random amplified polymorphic DNA.

Table 1. Site and cultivar information for 31 red clover seed fields grown in 1992 in the Willamette Valley, Oregon.

	Location				Seed source grouping†			
Site	County	Latitude	Longitude	Cultivar	Crop year	RAPD class	Certification class	Named cultivar
		° ′N	° ′W					
1	Marion	45 02	123 01	Marathon	1	1	1	1
2	Marion	45 11	122 55	Common‡	1	2	2	2
3	Marion	45 13	122 56	Atlas	2	1	1	1
4	Marion	45 14	122 57	Marathon	1	1	1	1
5	Clackamas	45 18	122 45	Common	2	2	2	2
6	Marion	45 14	122 52	Common	1	2	2	2
7	Clackamas	44 10	122 37	Kenland	1	2	2	1
8	Clackamas	45 08	122 41	Kenland	1	2	1	1
9	Clackamas	45 07	122 42	Kenland	1	2	2	1
10	Marion	45 04	122 44	Common	1	2	2	2
11	Marion	44 57	122 56	Kenland	1	2	2	1
12	Polk	45 03	123 13	Common	2	2	2	2
13	Polk	44 55	123 13	Kenland	1	2	2	1
14	Yamhill	45 06	122 13	Kenland	1	2	2	1
15	Yamhill	45 07	123 19	Kenland	2	2	2	1
16	Yamhill	45 05	123 25	Kenland	1	2	2	1
17	Yamhill	45 12	123 11	Hedges	1	2	2	1
18	Yamhill	45 21	123 10	Marathon	1	1	1	1
19	Yamhill	45 20	123 02	Common	1	2	2	2
20	Yamhill	45 09	123 02	Reddy	1	2	2	1
21	Multnomah	45 40	122 52	Common	1	2	2	2
22	Multnomah	45 44	122 50	Common	1	2	2	2
23	Washington	45 30	122 57	Common	1	2	2	2
24	Washington	45 41	123 13	Arlington	1	2	2	1
25	Washington	45 35	123 02	Common	2	2	2	2
26	Washington	45 34	123 02	Common	1	2	2	2
27	Washington	45 33	123 06	Common	1	2	2	2
28	Washington	45 30	122 58	Atlas	1	1	1	1
29	Washington	45 29	123 01	Leisi	2	2	2	1
30	Washington	45 29	123 01	Sapporo	1	2	1	1
31	Benton	44 33	123 15	Kenland	1	2	1	1

† Seed source groupings are based on: crop year (seed crop since establishment; 1 = first seed crop and 2 = second seed crop); RAPD class (two classes determined by random amplified polymorphic DNA analyses; 1 = Kenland and 2 = Marathon); certification class (seed fields being grown under the Oregon Seed Certification rules for number of years of generational advance from the foundation class; 1 = certified and 2 = noncertified classes); and named cultivar (seed produced is of a named cultivar; 1 = named cultivar and 2 = not a cultivar).

‡ Common designation unnamed, Oregon-adapted ecotypes.

samples where weighed and the percentage of dry mass determined to calculate total herbage dry mass per plot.

Neutron attenuation access tubes 1.5 m long were installed at each site 1 wk before the first herbage removal treatment was applied. The relative soil water content for each site was estimated by determining the average neutron attenuation count ratio at 38-, 60-, 90-, and 120-cm soil depths and multiplying the count ratio by the USDA-NRCS soil survey estimate of soil bulk density for the soil class at each site (1.2–1.55 g cm^{-1}). The amount of precipitation from rain or supplemental irrigation was measured using a volumetric gauge placed 1.5 m above ground level at each site. Neutron attenuation counts and precipitation were recorded during each visit to each site (approximately every 14–21 d beginning 7 April and ending 14 September). The relative seasonal change in soil water content for each site was calculated by adding the initial soil water measurement to all subsequent measurements that resulted in positive measurement changes from the previous recording date, and then subtracting the final soil water measurement at the time of seed harvest (Steiner et al., 1995).

The number of flowers in four 20 by 50 cm areas were counted at the same time the neutron attenuation measurements were made. The time of seed harvest ranged from 24 August to 15 September and depended on the time that all mature flower heads were dry and the florets would shatter if disturbed. All plots were harvested in the early morning when dew prevented shattering. Stand density was determined from the number of plants counted in a 1-m^2 frame in each plot. To estimate seed yield, all aboveground plant material from a 1 by 2 m strip was cut with a gasoline-powered mower, put into a burlap bag, air-dried at ambient temperature until 1 d before threshing, and then dried in a forced-air oven at 40°C for 12 h. The dried plant material from each plot was weighed (season-end phytomass yield), and the seeds were threshed using a belt thresher, cleaned, and weighed.

Root Health Evaluations

Twenty red clover plants were selected at random and excavated from the field area adjacent to the plot area at the time of application of the early herbage removal treatment and at the time of seed harvest. The root diameter from each plant was measured just below the crown and then bisected with a knife from the crown to the tip of the root. The degree of root rot was scored on a scale of 0 to 5, where 0 = completely healthy root tissue, 1 = presence of few superficial light-brown lesions affecting <20% of the taproot, 2 = presence of few superficial dark-brown lesions affecting 20 to 40% of the taproot, 3 = presence of numerous extended and profound dark-brown lesions affecting 40 to 60% of the taproot, 4 = presence of numerous extended and profound dark-brown lesions affecting 60 to 80% of the taproot, and 5 = >80% of the taproot tissue decayed or the plant is dead (Oliva et al., 1994b). The root halves were also examined for root borers, as indicated by the presence of insects or their tunnels, to determine the percentage of roots with root borer infestation (Steiner et al., 1997).

To determine the identity of the pathogen associated with root discoloration and decay, isolations were made from an additional five to 10 roots collected from each site soon after the time of seed harvest. Each root was rinsed under running tapwater for 1 to 2 min., blotted dry, and bisected lengthwise with a sterile scalpel. Tissue pieces 3 to 4 mm in diameter were removed with a sterile scalpel from the margin of healthy and discolored tissue, placed in a 100 mL L^{-1} bleach solution for 1 to 3 min., blotted dry on sterile absorbent tissues, and plated on water agar, potato dextrose agar (PDA), or Komadas medium (Dhingra and Sinclair, 1995). The plates were incubated at 20°C for 3 to 4 wk.

Pathogenicity of three suspect *Fusarium* isolates was determined by inoculation of 12-wk-old Kenlan red clover plants grown in 20-cm-diameter pots (two plants per pot) in a pasteurized greenhouse potting mix. A sterile dissecting needle was inserted three or four times into the crown of each plant to simulate root borer injury. One plant per pair of plants in each pot was inoculated by placing a 3- to 4-mm-diameter piece of PDA with suspect *Fusarium* at the point of inoculation on the crown. A sterile block of PDA was placed at the wound site on the second plant of each pot. Inoculations were made on each of five replicated plant pairs for each of the three isolates. The pots were arranged at random on a greenhouse bench and watered from above. After two weeks, both plants in each pot were bisected and examined for discoloration. Tissue pieces were removed from the margin of healthy and discolored tissue and cultured as described above and three of these isolates were submitted to the *Fusarium* Research Center, Department of Plant Pathology at Pennsylvania State University for species identification (P. Nelson, 1993, personal communication).

Random Amplified Polymorphic DNA and Cladistic Analyses

A random sample of leaves was collected from plants at the time of seed harvest for 30 of the 31 sites used in the study. A sample was not taken at Site 5 because all plants in the field had died between the time of herbage removal and seed harvest. The leaf samples were bulked, frozen in liquid N, lyophilized, and then ground into a fine powder at room temperature. Two replicates of 30 to 40 mg of ground material from plants collected at each field were used to distinguish cultivar differences by DNA analysis using random amplified polymorphic DNA (RAPD) analysis. The DNA was extracted, amplified, and analyzed as presented in Steiner et al. (1998). Operon (Operon Technologies, Alameda, CA) decamer primers OPA-06, 08, 09, 10, and 19; OPB-06, 07, 08, and 13; OPC-09 and 16; OPH-04, 05, and 12; and OPM-03 and 12 were screened for RAPD product band polymorphisms using leaf material collected from eight named cultivars (Arlington, Atlas, Cherokee, Hamidori, Kenland, Kenstar, Mammouth, and Marathon; and three locally adapted ecotypes from Oregon (Oregon Common 1 and Oregon Common 2) and Wisconsin (Wisconsin Common) that were grown in a field nursery near Corvallis, OR in 1994.[1] Suitable bands were selected if (i) an intense and unique band occurred in at least one of the cultivars examined, (ii) the band did not occur in all cultivars, and (iii) the band was repeatable in all replications (Steiner et al., 1998). Primers OPB-07 (GGTGACGCAG), OPB-08 (GTCCACACGG), and OPH-05 (AGTCGTCCCC) met the selection criteria and produced two, three, and seven polymorphisms, respectively.

The scored RAPD band data were used in a maximum parsimony analysis using a general heuristic search model that retained only minimal trees using PAUP software (Swofford, 1993). A total of 19 350 trees were examined and summarized by the 50% majority rule consensus method. Included with the 30 field site samples analyzed were reference samples of the cultivars Arlington, Atlas, Kenland, and Marathon, and the two locally adapted Oregon ecotypes (Oregon Common 1 and -2) that were grown in a nursery and that were analyzed for RAPD bands and scored the same as the field samples.

Statistical Analyses

A modified stability analysis (Hildebrand, 1984) was used to determine the uniformity of herbage removal time effects on seed yield across the range of environmental conditions represented by the 31 sites. Following Eberhart and Russell (1966), who used genetic sources as the experimental constant between locations, and as adapted for measuring agronomic practice effects by Steiner et al. (1995), the average seed yield for the two herbage removal times at each site was defined as the environmental index (EI) for that site. The greater the average seed yield at a site, the larger its EI. The seed yield (SY) for each herbage removal time was related to EI by the linear expression:

$$\mathrm{SY} = a + b\mathrm{EI} \quad [1]$$

where SY = yield for each haying time, and EI = the average seed yield for the two herbage removal time treatments at each site. Pearson's correlation coefficients, and linear and nonlinear regression analyses were used to determine functional relationships among variables.

All plant growth and root health data were analyzed by analysis of variance or covariance, depending upon the significance of the supplemental irrigation cofactor. When the contrast test for irrigation was not significant, the analysis of variance model used was:

$$Y = A + T + \mathrm{AT} + E \quad [2]$$

where Y is the dependent variable for each plant development or root health measure, A is seed crop stand age (first- and second-year seed crop); T is the time of herbage removal (early and late), and AT is the interaction of the A and T variable, and E is the within-error term with 54 degrees of freedom. When the irrigation contrast was significant, the analysis of covariance model:

$$Y = A + T + \mathrm{AT} + \mathrm{Covar\ I} + E \quad [3]$$

was used, where the independent variables are the same as in Eq. [2] except for the inclusion of Covar I$(X - \overline{X})$, which is the effect explained by the difference of the covariate (X) for irrigation (with or without supplemental irrigation) from its mean ($\overline{X}$), and with E having 53 degrees of freedom.

The effects of the three seed source groupings based on genetic similarity by RAPD analysis, seed certification class, and cultivar kind were determined using the analysis of covariance model:

$$Y = \mathrm{SS} + \mathrm{Covar\ I} + \mathrm{Covar\ J} + \mathrm{Covar\ K} + E \quad [4]$$

where SS is the effect of any of the three seed sources groupings, Covar I, J, and K are the effects of covariates for age of stand, time of herbage removal, and supplemental irrigation when these covariates were tested as significant.

Pearson correlation coefficients (r) were calculated to determine the associations among the plant growth and root health variables (Table 2). Multivariate factor analysis was used to classify the high degree of collinearity among the plant growth and root health variables. Separate factor analyses

[1] The use of trade names in this publication does not imply endorsement of the products named nor criticism of similar ones not mentioned.

Table 2. Pearson correlation coefficients among red clover characteristics that influence seed yield.

Parameters	E-Herbage	L-Herbage	E-SY	L-SY	E-Flow	L-Flow	E-Phyto	L-Phyto	WD	E-Dis	L-Dis	E-Diam	L-Diam	E-Borer	L-Borer	E-Stand
	r															
Early forage (E-Herbage)																
Late forage (L-Herbage)	0.03															
Early seed yield (E-SY)	0.18	0.65**														
Late seed yield (L-SY)	0.03	0.60**	0.93**													
Early flower density (E-FLow)	0.06	0.41*	0.59**	0.42*												
Late flower density (L-Flow)	0.13	0.47**	0.67**	0.79**	0.66**											
Early final phytomass (E-Phyto)	−0.13	0.67**	0.86**	0.78**	0.64**	0.64**										
Late final phytomass (L-Phyto)	−0.14	0.53**	0.80**	0.86**	0.58**	0.67**	0.83**									
Water depletion percentage (WD)	−0.50**	0.28	0.69**	0.57**	0.30	0.47**	0.64**	0.65**								
Early root disease (E-Dis)	−0.11	−0.13	−0.31	−0.14	−0.14	−0.01	−0.31	−0.04	−0.11							
Late root disease (L-Dis)	−0.07	−0.14	−0.37*	−0.21	−0.23	−0.12	−0.34*	−0.12	−0.20	0.89**						
Early root diameter (E-Diam)	0.01	0.13	0.03	0.22	0.08	0.16	0.07	0.07	−0.01	0.60**	0.57**					
Late root diameter (L-Diam)	0.05	−0.03	−0.17	0.10	0.00	0.14	−0.14	−0.14	−0.16	0.72**	0.57**	0.72**				
Early root borer (E-Borer)	−0.38*	−0.18	−0.34*	−0.23	−0.32	−0.15	−0.31	−0.15	0.02	0.64**	0.63**	0.32	0.42*			
Late root borer (B-Borer)	0.06	−0.34*	−0.62**	−0.45**	−0.43*	−0.27	−0.58**	−0.40*	−0.41*	0.59**	0.75**	0.21	0.35*	0.66**		
Early stand density (E-Stand)	0.07	0.01	0.16	0.01	0.30	0.10	0.28	0.10	0.20	−0.61**	−0.50**	−0.49**	−0.68**	−0.45**	−0.46**	
Late stand density (L-Stand)	−0.20	0.23	0.44*	0.18	0.43*	0.21	0.44**	0.25	0.42*	−0.66**	−0.65**	−0.59**	−0.69**	−0.46**	−0.62**	0.79**

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

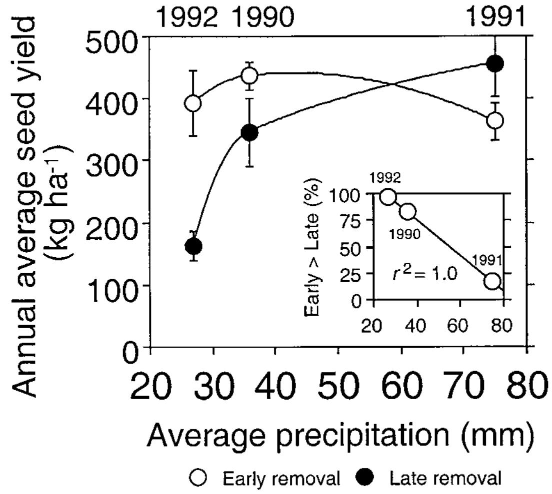

Fig. 1. Comparison of the effects of herbage removal time and average annual precipitation amount on average annual red clover seed yield from 31 sites in 1992 with yields at 12 sites previously reported in 1990 and 1991. The insert figure shows the effect of average annual precipitation on the percentage of sites that have higher seed yields with early than late herbage removal time.

were done for the early and late herbage removal treatments. Rotated loading values were calculated by the varimax rotation method (Manly, 1986) with the model forced to use two factors using SYSTAT 5.2 for the Macintosh (1992). Rotated loading values greater than the absolute value of 0.2 were generally considered significant, unless Pearson's r for any pairwise comparison of two variables was not significant when validating the definition of factors. All reported differences are significant at $P \leq 0.05$, unless otherwise stated.

RESULTS

Site and Herbage Removal Time Relationships

In 1992, average seed yields were greater for the early (410 kg ha^{-1}) than the late (315 kg ha^{-1}) herbage removal treatment. To validate the 1992 herbage removal time effects, comparisons were made with similar herbage removal time treatments reported from 1990 and 1991 (Steiner et al., 1995). In the earlier study, delaying herbage removal time at high-yield-potential sites increased seed yield, while conversely, early herbage removal was beneficial at low-yield-potential sites. Much of the difference in seed crop response to time of herbage removal was explained by the amount of water received by the crop (from natural precipitation or overhead sprinkler irrigation) following herbage removal.

The average annual precipitation amount received from the time of herbage removal until seed maturity in 1992 was 27 mm compared with 36 and 75 mm in 1990 and 1991, respectively. The average annual seed yield for the early herbage removal treatment was more stable (not influenced by the amount of water received in any year) than the late herbage removal treatment (Fig. 1). Only in the wettest of the 3 yr (1991) was there a benefit from the delayed herbage removal treatment. For the late herbage removal treatment, seed yield declined as the amount of water decreased from the time of herbage removal until seed maturity. When <30 mm

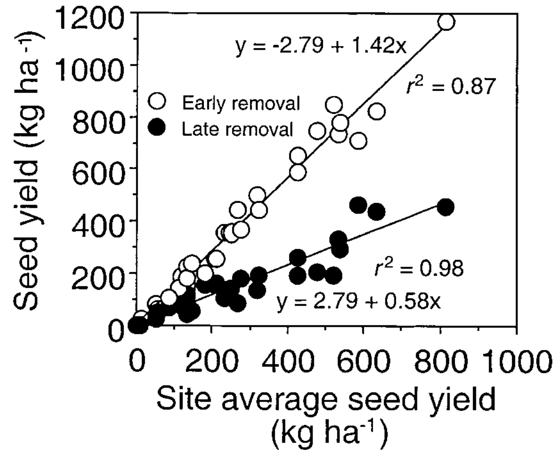

Fig. 2. Stability analysis for early (early May) and late (late June) herbage removal treatments used with red clover seed yield grown in 1992 at 31 sites. Site average seed yield is equal to the average of the early and late herbage removal phytomass yields at each location.

of precipitation were received (as in 1992), there was no compensation for delayed herbage removal at highly productive sites and none of the fields had seed yields greater than the early treatment (Fig. 2). In contrast, 83% of the sites in 1990 responded best to early herbage removal and 17% of the sites in 1991. Also in 1992, there was no association between the amount of water received at each site and seed yield (Fig. 3A). The responses to early and late herbage removal time in 1992 fit the general findings for the same treatments in 1990 and 1991, based on differences in precipitation among the 3 yr of study.

Even though the irrigated sites on average yielded more than all nonirrigated sites, the yields at the irrigated sites did not differ from the best nonirrigated site yields. Two of the five irrigated sites (Sites 4 and 34) had 153 and 145 mm of water applied by overhead sprinklers, respectively, but these did not have greater seed yields than the other three irrigated sites (Sites 11, 21, and 32) that received only 32, 36, and 27 mm of irrigation water, respectively ($P \leq 0.69$).

Even though plants grown at irrigated sites did not necessarily yield more than nonirrigated sites, a greater percentage of available soil water was depleted (12 and 23%, respectively) and more nonreproductive phytomass was produced following herbage removal than nonirrigated sites (4852 and 2953 kg ha^{-1}, respectively). As a result, seed yield was not related to the amount of water received after the time of herbage removal, but was positively associated with soil water depletion percentage (Fig. 3B).

Root Health Characterization

In this study, *Fusarium solani* was isolated from 19 of 28 sites that had viable roots sampled at the time of seed harvest. No *Fusarium* isolates were found at nine of the 28 sites, including six sites that had no organisms and three that had unidentified organisms other than *Fusarium*. At the remaining three of the total 31 sites, the plants were dead at harvest time, so no isolations

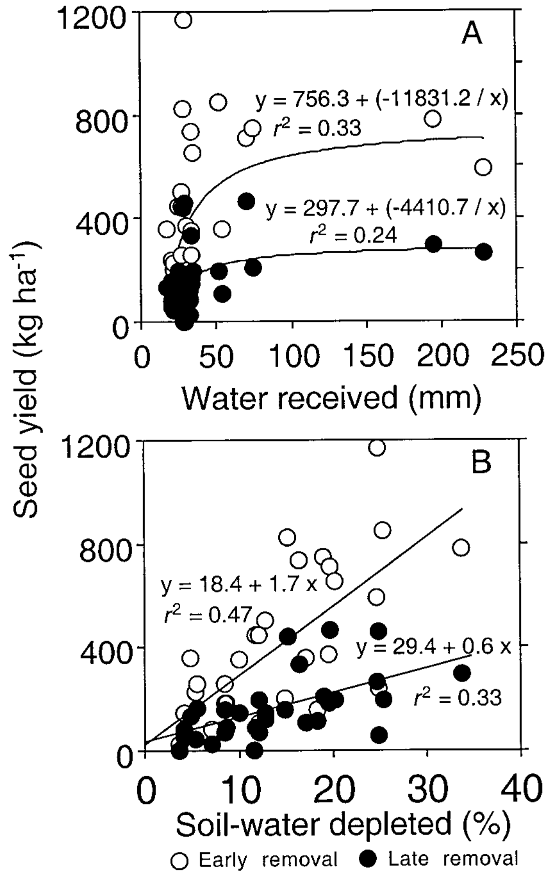

Fig. 3. The effect of herbage removal time and (A) amount of total water received, and (B) percentage of soil-water depleted on red clover seed yield in 1992 at 31 sites.

were attempted from the roots. When isolates of *F. solani* were inoculated into greenhouse plants, symptoms of discoloration and decay typical of those found in the original roots in the absence of root borer damage were reproduced. Only two of the fifteen paired control plants displayed any discoloration (probably due to splash contamination during watering) with all other control plants symptom free.

The severity of season-end root rot damage across all sites was linearly dependent on the amount of disease incidence at early-herbage removal time (Fig. 4A). The increase in root rot incidence between the time of early herbage removal and seed harvest was ≈0.85 index units, regardless of the initial root rot rating. Root rot damage was greater in second-year than first-year crop stands (rating indexes of 3.4 and 1.4, respectively), and was unaffected by irrigation (Table 3).

In addition to root discoloration and decay caused primarily by *F. solani*, variation in the percentage of roots infested with root borers was also noted. The root borer infestation at the time of early herbage removal varied, but increased from the time of early herbage removal until seed harvest at 21 of 31 sites (Fig. 4B). Borers were responsible for tunneling into root and crown tissue, and causing extensive damage. One to several borers could be found in a single infested root. Even at sites with no measured early-season infestation,

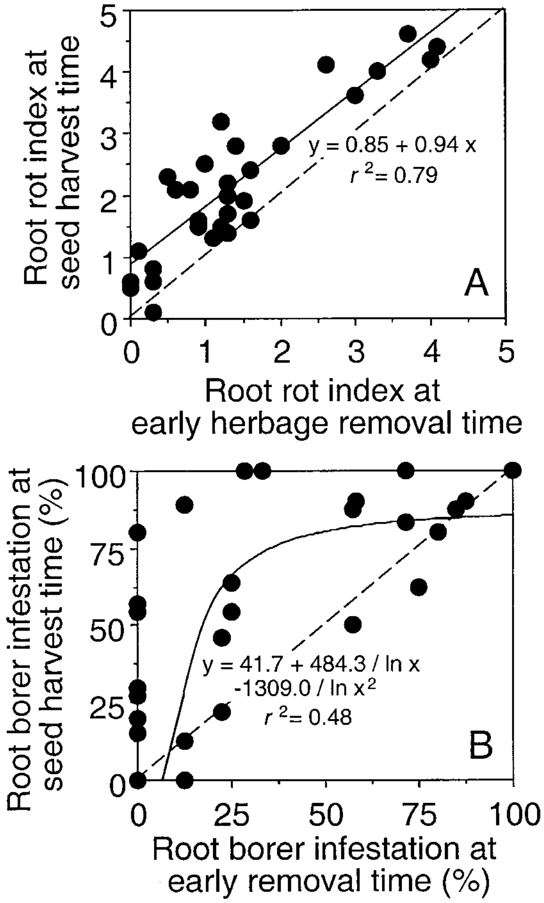

Fig. 4. The relationships of (A) root rot severity, and (B) root borer infestation and red clover seed production with early herbage removal and seed harvest times when grown in 1992 at 31 sites. Data are fitted with nonlinear regression functions. Dashed lines indicate the 1-for-1 line relationship for both variables.

the percentage of roots infested with root borers had increased rapidly by the end of the season (range root borer incidence at the time of seed harvest for sites with no initial infestation was 0 to 80%). Early and late root rot ratings were correlated respectively with early and late root borer infestation percentage, ($r = 0.64$ and 0.75, respectively). The most rapid change in root borer infestation occurred in plants that had an initial infestation of $\leq$25% at the time of early herbage removal (Fig. 4B). Stands in the second seed year had greater average root borer infestation than first-year stands (67 and 37%, respectively).

Crop Genetic Relationships and Root Health

The fields were divided into two classes based on maximum parsimony analysis of 12 RAPD polymorphic bands (Fig. 5). Samples from the three fields of Marathon and two of Atlas were grouped into a well-resolved tree topology that was supported by the inclusion of two cultivar standards. This group was designated as Marathon-type. Marathon was selected from polycrosses among several cultivars and included Arlington, but not Kenland (Smith, 1994). The remaining fields (20 of the 30 sites) were predominately composed of locally adapted ecotypes and had poor tree topology. These were designated as Kenland-types A and B because among them were the Kenland cultivar standard and two certified Kenland seed fields. Also included among the Kenland-types were the cultivar standards for Arlington, two locally adapted ecotypes that were maintained by private seed companies for trade in the noncertified seed market (Oregon Common 1 and Common 2), and samples from certified fields of the cultivars Leisi, Reddy, and Sapporo. Oregon Common 1 was closely related to the Kenland standard, verifying its reported original selection from a Kenland seed field in Oregon (R. Peschka, personal communication, 1995). Arlington was selected from polycrosses among several cultivars including Kenland (Smith et al., 1973). There are no published reports available regarding the genetic backgrounds of Leisi, Reddy, Sapporo, and Hedges.

Marathon-type cultivars, certified fields, and named cultivars were all more resistant to root borer infestation than the Kenland-types, noncertified fields, and common seed sources, respectively (Table 4). However, root rot disease infection level and seed yield were unaffected by the seed source classifications. There were no root rot differences between the Kenland Type-A and Type-B groups (data not shown), so these two groups are further analyzed together as a single group (Kenland-type) and compared with the group Marathon-type.

DISCUSSION

Root rot has been shown to greatly reduce the capacity of red clover plants to produce seed, particularly when soil water availability is limited during the second year of seed production (Oliva et al., 1994b). In this experiment, the effects of root health on age of stand, time of herbage removal, and genetic background of red clover cultivars on root health in seed production systems were found to be complex.

The general poor tree topology for the Kenland-type samples indicated limited genetic divergence has occurred among the seed sources (Wolfe and Liston, 1998). This suggests that the different seed lots are probably members of a common genetic pool (Furman et

Table 3. Analysis of variance and covariance based on significant supplemental irrigation effects for two seed crop ages and two times of forage removal of red clover grown in 1992 at 31 locations in western Oregon.

Variable	Degrees of freedom	Herbage dry mass	Flower density	Seed yield	Root rot	Root borer	Root diameter	Stand density	Season-end phytomass
		P†							
Age (A)	1	0.209	0.789	0.537	0.001	0.006	0.001	0.001	0.626
Time (T)	1	0.004	0.001	0.006	0.015	0.028	0.001	0.553	0.002
$A \times T$	1	0.706	0.912	0.454	0.742	0.787	0.702	0.880	0.268
Irrigation (covariate)	1	ns	ns	0.007	ns	ns	0.001	ns	0.001

† Probability value indicates the significance of F according to analysis of variance or covariance. ns indicates that the irrigation covariate was not significant at $P \leq 0.05$, so analysis of covariance was not used.

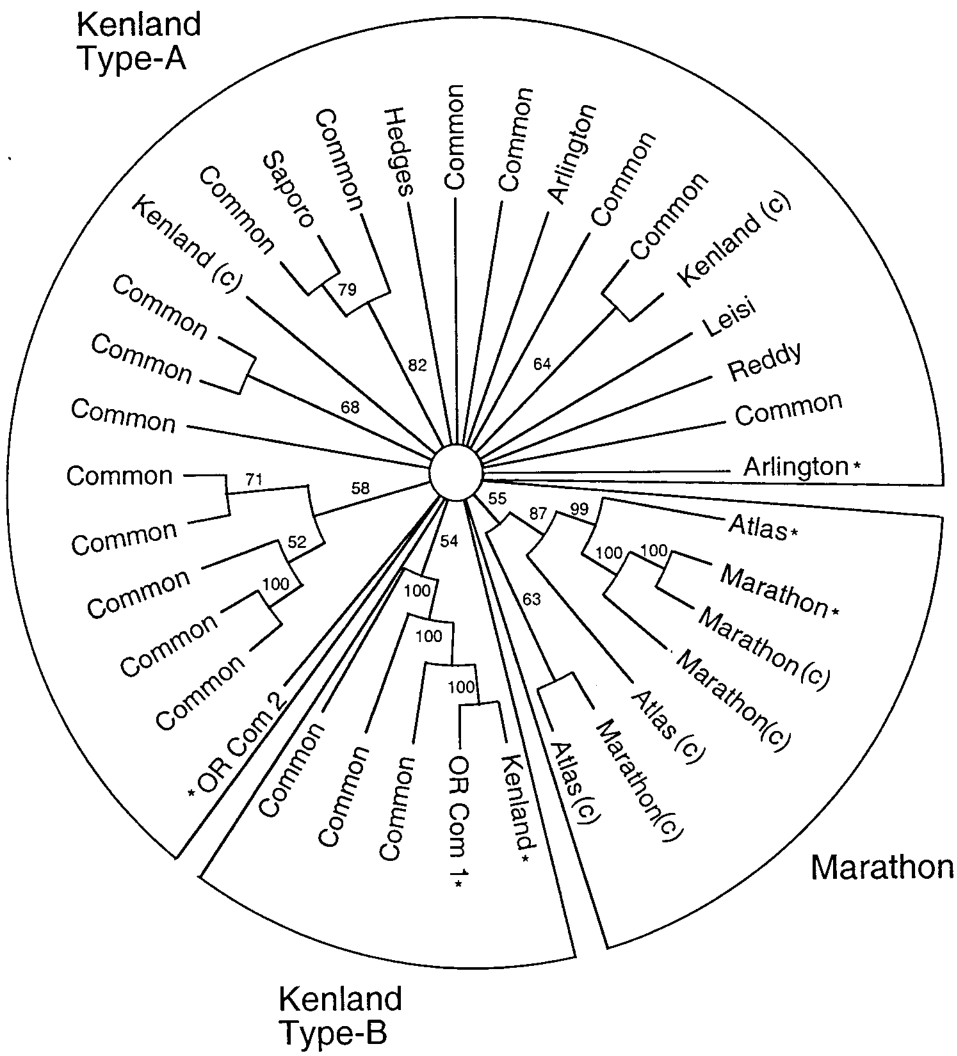

Fig. 5. Maximum parsimony analysis using a general heuristic search model of 12 random amplified polymorphic bands to determine the relationships among 30 commercial red clover seed fields and six known cultivar seed sources. Cultivars identified with an "*" indicate the known cultivar sources. The name "Common" indicates seed sources that are of unknown cultivar origin. Cultivar names followed with "(c)" indicate seed fields that are grown under the rules of the Oregon Seed Certification program. The numbers along branches indicate branch lengths.

al., 1997), which would be expected of different seed lots from a single cultivar source. Pedigree information (Taylor and Quesenberry, 1996) and the cladistic analysis results (Fig. 5) support the general absence of a directed gene flow (Van Heusden and Bachmann, 1992; Van Buren et al., 1994) among many of the seed sources used for seed production in this experiment. Most red clover cultivars have been developed from similar genetic progenitors (Taylor and Quesenberry, 1996). The uniqueness of Marathon from the Kenland-types may

Table 4. Comparisons of red clover root health and seed yield responses on the basis of seed source classification by genetic similarity, seed certification class, and cultivar kind. Results are from replicated plots in 31 western Oregon fields grown in 1992 that were subjected to early and late herbage removal treatments. Contrast comparisons are based on analysis of covariance of significant crop age, time of herbage removal, and supplemental irrigation covariants.

	RAPD†			Certification‡			Named cultivar§			Covariate		
Variable	1	2	Contrast	1	2	Contrast	1	2	Contrast	Age	Time	Irrig.
			$P \leq$			$P \leq$			$P \leq$			
Disease (0–5)	1.6	1.8	0.51	1.8	1.8	0.91	1.7	1.9	0.45	***	***	ns
Root borer (%)	24.5	46.2	0.05	30.7	46.9	0.08	36.2	53.1	0.04	**	**	ns
Seed yield (kg ha^{-1})	321.5	268.5	0.49	306.3	266.8	0.53	261.8	301.0	0.51	ns	***	**
Field locations (n)	5	26		8	23		19	12				

,* Significant at the 0.01 and 0.001 levels of probability, respectively. ns is not significant.

† RAPD (random amplified polymorphic DNA) classes based on the groupings of seed fields from the results shown in Fig. 1 with 1 indicating Marathon and 2 indicating all other Kenland types.

‡ 1 indicates seed crop produced under the rules of seed certification, and 2 indicates noncertified cultivars and common seed classes.

§ 1 indicates named cultivar, and 2 indicates common seed fields without cultivar designation.

Table 5. Rotated loading values from a varimax rotation factor analysis of seven measures of root and crown health and five measures of crop development capacity from red clover grown for seed at 31 sites in western Oregon in 1992.

Parameters	Factor name: Root health	Factor name: Plant growth capacity
	— Rotated loading values —	
Early herbage removal treatment		
Early-root disease	0.927*	−0.130
Late-root disease	0.846*	−0.246*
Late-root diameter	0.781*	0.011
Early-root diameter	0.706*	0.190
Final stand density	−0.678*	0.144
Early-root borer	0.657*	−0.210*
Late-root borer	0.583*	−0.583*
Seed yield	−0.179	0.929*
Season-end phytomass	−0.176	0.909*
Soil-water depletion	−0.048	0.679*
Flower density	−0.129	0.600*
Herbage yield	−0.142	−0.220*
Variance explained by factor (%)	34	26
Late herbage removal treatment		
Early-root disease	0.911*	−0.060
Late-root disease	0.876*	−0.174
Late-root diameter	0.797*	0.156
Final stand density	−0.779*	0.248
Early-root diameter	0.718*	0.289
Late-root borer	0.649*	−0.467*
Early-root borer	0.629*	−0.184
Season-end phytomass	0.045	0.963*
Seed yield	−0.048	0.940*
Flower density	0.014	0.784*
Soil-water depletion	−0.165	0.593*
Herbage yield	−0.094	0.576*
Variance explained by factors (%)	35	30

* Significant at the 0.05 level of probability after verification. Pearson correlation coefficients shown in Table 1.

be due to Marathon being an advanced-generation synthetic cultivar that includes parental types genetically divergent from Kenland (Smith, 1994). Pedigree information describing the origins of Atlas were not available, but this cultivar is known to have been developed by a private company in Minnesota, in an ecogeographic region similar to Wisconsin, where Marathon was developed (R. Smith, 1996, personal communication). The RAPD analysis suggested Atlas has a similar genetic parentage as Marathon, but is different from Arlington. Both of the Arlington samples from the commercial seed fields as well as the nursery were placed in the Kenland-type group. The greater root borer resistance of Marathon-type cultivars, certified fields, and named cultivars compared with the Kenland-types, noncertified fields, and common seed sources, respectively (Table 4), indicated that genetic selection for stand persistence can be used to improve performance.

Numerous correlations among the different plant-measured variables made the interpretation of the effects of herbage removal time less than straightforward (Table 2). Because of collinearity, commonality among the various plant and root health measurements was determined using multivariate factor analysis to identify the underlying structural relationships (Table 5). Two general factor groupings were identified in response to the early or late herbage removal treatments. The first factor (root health) was comprised of measures for root disease infection, root borer infestation, root diameter, and stand density at harvest time. Significant factor analysis rotated loading values (loading values) were found for the variables in the root health factor in both the early and late herbage removal treatments. Stand density was inversely correlated with all measures of root health, regardless of time of herbage removal (Table 2). The second factor (plant growth capacity) was comprised of variables related to plant measurements and included herbage yield, flower head density, season-end phytomass, seed yield, and total soil water depletion amount. All of these variables had significant loading values, regardless of herbage removal time.

For the early herbage removal time treatment, the plant growth capacity variables were associated with late sampling time root disease and, regardless of early or late sampling time, root borer percentage. Seed yields from the early herbage removal time were related to the general capacity of the plants to produce nonreproductive phytomass (r = 0.86) and flowers following herbage removal (r = 0.59), and to utilize available soil water (r = 0.69). The seed yield responses to root disease (r = −0.37) and root borer infestation (r = −0.34) at the time of harvest were less than with the other variables mentioned above. When the late herbage removal time treatment was applied, only season-end root borer percentage was grouped with the plant growth capacity variables (Table 5).

Seed yield for the late-herbage removal time treatment was highly influenced by the capacity of plants to produce nonreproductive phytomass following herbage removal (r = 0.86) and to produce flowers (r = 0.79). Delayed herbage removal placed flower development at a time when water availability was beginning to become limited due to low precipitation amounts at that time and because more water was utilized to support the herbage before removal than for growth recovery following herbage removal (Steiner et al., 1995). Plants subjected to early herbage removal were not exposed to as much stress because flowering was initiated at an earlier time than for the late removal time. Because soil water was not as limited at the time of early herbage removal, herbage yields for the early and late removal time treatments were not correlated (r = 0.03), and there was no relationship between early removal time herbage yield and its resulting season-end phytomass (r = −0.13).

For those fields that had irrigation water applied, the application amounts were less than 60% (data not shown) of the 280 to 340 mm recommended for optimal seed yield (Oliva et al., 1994a). This indicates that farmers who have irrigation available should have used greater application amounts than used in these field examples. Using the recommended water application amount resulted in less root rot severity and higher seed yields in the second year of seed production than for nonirrigated or 50% of optimal application conditions (Oliva et al., 1994b).

Factors associated with the ability of plants to extract soil water, rather than the amount of soil water available to plants, influenced red clover reproductive development and seed yield following herbage removal. Since seed yield was generally unaffected by seasonal differences in the amount of precipitation received when early herbage removal was used (Fig. 1), then sufficient amounts of available soil water and adequate plant uptake mechanisms were in place to produce flowers and allow complete seed maturation. The amount of soil water depleted by the plants after herbage removal can be considered as an indicator of root integrity and was correlated with the amount of season-end phytomass produced (r = 0.64 and 0.65, respectively). This was supported by the finding that fields that produced the greatest amount of herbage also had the greatest amount of final phytomass production (r = 0.53). More flowers are produced when plants recover rapidly from herbage removal than for plants with poor root function, and proper soil water replenishment by irrigation can counteract the negative effects of root rot disease (Oliva et al., 1994b). Also, in an earlier study (Steiner et al., 1995), the percentage of soil water depleted by plants was also positively related to seed yield.

Most significant was the finding that, regardless of herbage removal time, there was no relationship between root rot disease level and seed yield (r = −0.31, and −0.21, respectively) but that the percentage of roots infested with root borer was inversely related to seed yield (r = −0.62 and −0.45, respectively). In forage systems, the root borer has also been shown to be a primary causal agent of stand decline (Jin et al., 1992), to reduce herbage yields (Pruess and Weaver, 1958), and to intensify the severity of root rot disease damage (Leath and Byers, 1973). Under the conditions for seed production systems in western Oregon in 1992, the differences due to genetic background of seed fields were attributed to differences in root borer infestation and not disease infection (Table 4). Therefore, an alternative strategy for improving persistence in red clover seed fields may be to develop cultivars that are resistant to the root borer rather than focusing on root disease resistance, which has been the primary strategy used to improve cultivars for forage use. Since selection for root rot resistance is pathogen-specific for the region of production (Steiner et al., 1998), the efficacy of selection for *F. solani* resistance vs. root borer resistance needs to be determined. Any selection strategy for improving red clover root health should also include selection for high flower density to ensure cultivar acceptance by seed growers.

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